

Lentivirus qPCR Quantification Kit

(Catalog # K1471-100 Rxns; Store at -20°C)

I. Introduction:

BioVision's Lentivirus qPCR Quantification Kit is used for the quantification of Lentivirus. This kit employs a quick RNA extraction step that can be conveniently and directly coupled with a qRT-PCR reaction. It is a simple one step titration assay designed to deliver high sensitivity and specificity. In this kit, the threshold cycle (C_t) values of the viral lysate and the lentiviral standards provided are determined by qRT-PCR. The C_t values are then used to calculate the lentiviral titer of the virus samples.

II. Application:

- An ideal tool to quantify Lentivirus

III. Key Features:

- Reliable and Ready-to-use
- Results ready in less than 2 h
- High specificity** and sensitivity
- Minimal non-specific background

IV. Sample Types:

Virus-producing cell lines or purified viral preparations

V. Kit Contents:

Components	K1471-100 (100 Rxns)	Part Number
Virus Lysis Buffer	800 μ l	K1471-100-1
Reagent Mix	1.0 ml	K1471-100-2
STD1 (Lentivirus Standard 1)	300 μ l	K1471-100-3
STD2 (Lentivirus Standard 2)	300 μ l	K1471-100-4

VI. User Supplied Reagents and Equipment:

- Dye-based 2X qPCR MasterMix
Note: Please select the MasterMix appropriate for your specific instrument. Refer to **BioVision's Jade™ Master Mixes** (Cat. Nos. M1105-M1108) which come in a range of formulations. Each MasterMix has been optimized for performance according to the QPCR machine and reference dye (Mix (No dye) = without reference dye (ROX) dye, IcyCycler = for IcyCycler, low ROX = for low ROX, ROX = regular ROX). Please click the link. <https://www.biovision.com/documentation/support/QPCR-Selection-Guide.pdf> for selecting the appropriate MasterMix.
- qPCR Thermal Cycler
- PCR tubes
- 1X Phosphate Buffered Saline

VII. Shipping and Storage Conditions:

The kit should be stored at -20°C upon arrival. Avoid repeated freeze and thaw cycles. All reagents are stable for up to 12 months when stored properly at -20°C.

VIII. Assay Protocol:

Virus samples originating from both virus-producing cell lines or purified viral preparations can be used for determining the titer. The Virus Lysis Buffer provided in the kit helps to extract the viral RNA from the virus sample (the extracted RNA is then referred to as "viral lysate"). The Reagent mix and the 2X qPCR MasterMix (not provided) are then used with the viral lysate and the Lentiviral Standards (STD1/STD2) to determine the threshold cycle (C_t) values by qRT-PCR. The resulting C_t values are then used to calculate the lentiviral titer of the virus samples.

- Sample Preparation:** For purified viral samples: dilute the **virus** to the range of 10^6 - 10^8 pfu/ml with 1X **Phosphate Buffered Saline** prior to subjecting the virus to viral lysis. For viral samples directly from a virus producing cell line: collect the culture medium and centrifuge it for 5 min at 2000 g to remove cells/debris. The resulting supernatant will be subjected to viral lysis.
- Viral Lysis:** Add 2 μ l of the diluted purified viral preparation or the supernatant (from step 1) to 18 μ l of **Virus Lysis Buffer** and incubate at room temperature for 3 min. This solution is now referred to as the viral lysate. The C_t value of the viral lysate will be used to determine the titer of the unknown viral sample. Note: Please note in this step the viral sample has been diluted 1:10; therefore this dilution factor needs to be taken into consideration when calculating the titer.
- qRT-PCR Set-up:** Set-up the reactions as shown below with 2X qPCR Mastermix (not provided) in this kit. Please note that all reactions except Non Template Control (NTC), must be set-up in triplicates.

Components	Viral Lysate	STD1	STD2	NTC
2X qPCR Mastermix	12.5 μ l	12.5 μ l	12.5 μ l	12.5 μ l
Viral Lysate (from step 2)	2.5 μ l	-	-	-
STD1	-	2.5 μ l	-	-
STD2	-	-	2.5 μ l	-
Reagent-mix	10 μ l	10 μ l	10 μ l	10 μ l
Final volume per reaction	25 μ l	25 μ l	25 μ l	25 μ l
Total number of reactions	3	3	3	1

- qRT-PCR Program:** Program the real-time qPCR instrument as shown below. (Note: this specific program was optimized for ABI® StepOne™ system).

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Step	Temperature	Duration	Number of Cycles
Reverse Transcription	42°C	20 min	1
Enzyme Activation	95°C	10 min	1
Denaturation	95°C	15 sec	30
Annealing/Extension	60°C	1 min	

IX. Data Analysis:

The titer of your sample(s) can be calculated from C_t values by using the following formula:

$$\text{Titer of viral lysate} = 5 \times 10^7 / 2^{3(C_{ix} - C_{t1}) / (C_{i2} - C_{t1})}$$

C_{ix} = Average of 3 C_t values of the unknown sample

C_{t1} = Average of 3 C_t values of STD1

C_{i2} = Average of 3 C_t values of STD2

To calculate the titer of the unknown sample, be sure to include the dilution factor in the calculation. For eg. if you diluted the purified viral sample 1:100 in Step 1 and 1:10 in step 2 (default), then the titer of the unknown sample should be calculated as:

$$100 \times 10 \times 5 \times 10^7 / 2^{3(C_{ix} - C_{t1}) / (C_{i2} - C_{t1})}$$

Units: The final titer value obtained is in IU/ml.

X. Recommendations for Optimal Results:

1. Start the titration assay as soon as the virus is produced or thawed.
2. Upon receipt, make aliquots of all the components to minimize freeze-thaw cycles and contamination of reagents.
3. Always keep reaction components and mixtures on ice during set-up. Start the qPCR as soon as the reaction mixtures have been prepared.

XI. Related Products:

Product Name	Cat. No.	Sizes
Instant Lentivirus Detection Card	K1470	-10, -20 tests
Mag-Lentivirus and Retrovirus Purification Kit	K1458	20, 100 Preps
Lentivirus Mini Purification Kit	K1305	-10, -20 preps
Lentivirus Maxi Purification Kit	K1306	-2, -4, -10 preps
PEG Virus Precipitation Kit	K904	-50, -200 preps
Mag-Adenovirus Purification Kit	K1459	-10, -200 preps
Adenovirus Mini Purification Kit	K1300	-10, -20 preps
Adenovirus Maxi Purification Kit	K1301	-2, -4, -10 preps
Retrovirus Mini Purification Kit	K1307	-10, -20 preps
Retrovirus Maxi Purification Kit	K1308	-2, -4, -10 preps
HCV Mini Purification Kit	K1309	-10, -20 preps
HCV Maxi Purification Kit	K1310	-2, -4, -10 preps

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